

The Utility of a Single Sputum Specimen in the Diagnosis of Tuberculosis*

Comparison Between HIV-Infected and Non-HIV-Infected Patients

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Study objectives: (1) To assess the utility of a single sputum specimen in the evaluation of HIV-infected patients who are suspected of having tuberculosis (TB). (2) To identify radiographic findings that discriminate between HIV-infected patients with TB and those with pneumonia of other causes.

Design: Retrospective cohort analysis.

Patients: All patients evaluated at Harborview Medical Center, Seattle, between January 1986 and July 1994 in whom culture of respiratory secretions grew *Mycobacterium tuberculosis* or *Mycobacterium avium-complex*. Patients who were coinfecting with HIV formed the primary study group. Their chest radiographs were then compared with those of a matched group of patients with pneumonia of other causes.

Measurements and results: We identified 164 patients with TB, 20 of whom were HIV infected. The initial sputum specimen grew *M tuberculosis* in all HIV-infected patients and 99% of non-HIV-infected patients. Seventy percent of HIV-infected and 71% of non-HIV-infected patients had at least one positive smear. Most of these patients tested positive on their initial smear, and no significant difference was found between HIV-positive and HIV-negative patients (79% and 90%, respectively [$p=0.34$]). The addition of a second sputum smear identified all HIV-infected patients and all but one in non-HIV-infected patients who were ultimately determined to be smear positive. A total of 27 HIV-infected patients had a positive acid-fast bacilli sputum smear during the study period, 14 of which were attributable to TB (specificity=52%). The only radiographic findings that discriminated between HIV-infected patients with TB and those with pneumonia of other causes were the presence of cavitation or a miliary pattern ($p=0.014$).

Conclusions: A single sputum specimen was sufficient to establish the diagnosis in all HIV-infected patients with pulmonary TB. A single negative sputum smear made the diagnosis of TB significantly less likely. However, a minimum of two smears were necessary to achieve an acceptable early diagnostic yield. The presenting chest radiograph failed to discriminate between HIV-infected patients with TB and pneumonia of other causes in most cases.

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Key words: acid-fast bacilli; AIDS; respiratory isolation; sputum smear; tuberculosis

Abbreviations: AFB=acid-fast bacilli; MAC=*Mycobacterium avium* complex; TB=tuberculosis

Patients who are infected with HIV appear to be at unique risk for the development of pulmonary tuberculosis (TB).^{1,2} The diagnosis of TB may be obscured in this patient population by the nonspe-

cific nature of presenting symptoms and the absence of typical radiographic findings.³ Accordingly, clini-

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cians must maintain a high index of suspicion for TB when evaluating their HIV-infected patients with respiratory complaints. When these patients require hospitalization, a strong point can be made for placing them in respiratory isolation until the public health risk that they pose can be assessed.¹⁻⁴ The rapid assessment of this risk is currently limited to

the microscopic examination of expectorated sputum. Studies of serial sputum acid-fast bacilli (AFB) smears have demonstrated that the yield increases with each specimen that is examined.^{5,6} The commonly recommended practice of obtaining first morning sputa on three consecutive days⁷ is designed to maximize the yield while minimizing the time and resources required to achieve it. In our institution, we evaluate more than 30 HIV-infected patients with pneumonia for each one that is demonstrated to have TB. The patient discomfort and costs associated with this approach have led us to consider an alternative strategy, one that relies on fewer sputum specimens. We undertook this retrospective analysis to determine whether a single sputum specimen is sufficient to establish the diagnosis of TB in HIV-infected patients. Special emphasis was placed on the sensitivity and specificity of the sputum smear for the early diagnosis of TB. Acknowledging that the smear would be an imperfect diagnostic tool, we attempted to identify radiographic variables that would aid in the distinction between HIV-infected patients with TB and those with pneumonia of other causes.

MATERIALS AND METHODS

Patient Selection

We retrospectively reviewed mycobacterial laboratory reports at Harborview Medical Center, Seattle, a 330-bed county hospital, issued between January 1, 1986 and July 7, 1994. The study group consisted of all patients whose cultures grew *Mycobacterium tuberculosis* during this time period. Pulmonary TB was diagnosed when *M tuberculosis* was cultured from sputum, BAL, pleural fluid, or lung tissue. Patients were classified as being infected with HIV if they were identified by a computerized search of medical records using the ICD-9 discharge codes for HIV infection and AIDS, or if they were identified as patients of the Harborview-affiliated AIDS clinic, the largest clinic of its kind in the Pacific Northwest. HIV-infected patients with pneumonia of other causes were identified by searching hospital discharge records using the ICD-9 codes for HIV infection, AIDS, and pneumonia, excluding TB. During the course of the study, three HIV-infected and 27 non-HIV-infected patients presented with recurrent *M tuberculosis* infection. We report data only from their original presentation. In addition, we reviewed the records of all HIV-infected patients whose cultures grew *Mycobacterium avium* complex (MAC) during the study period.

This study was approved by the Human Subjects Committee at the University of Washington. Data were collected and analyzed using software (Microsoft Excel; Redmond, Wash). Statistical analysis was achieved using a two-tailed Student's *t* test.

Mycobacteriology

All sputum specimens that were at least 1 mL in volume were accepted for laboratory analysis. Our microbiology laboratory does not discern between spontaneously expectorated and in-

duced sputum specimens. However, Harborview's sputum induction policy allows us to state with confidence that the overwhelming majority of specimens were derived from spontaneously expectorated sputum. Sputum induction was not introduced in this institution until 1990 (halfway through this retrospective analysis). When it was introduced, it was used solely as a means of establishing the diagnosis of *Pneumocystis carinii* pneumonia. It was not until after the study period that the hospital infection control committee endorsed the use of this procedure in the evaluation of patients suspected of having TB.

Specimens were processed in the usual fashion.⁸ Acid-fast stains were performed using the auramine fluorochrome and Kinyoun methods, as has been described previously.^{9,10} The entire area of each auramine smear was examined at 250 x magnification by a trained technician. A smear was considered positive if either staining method demonstrated three or more AFB per slide. Acid-fast smears were further classified according to the American Lung Association standards.¹¹ Mycobacterial isolates were speciated in accordance with Centers for Disease Control standards.¹²

Radiography

Chest radiographs were interpreted by a pulmonary physician (C.D.B.), and this interpretation was compared with that recorded in the chart copy of the staff radiologist's report. When differences were identified, a third reading was obtained by a second staff radiologist who served as the tiebreaker on the finding in question. All interpretations were made without knowledge of previous interpretations. Infiltrates were classified as focal if they were confined to a single lobe or contiguous lobes; those involving two or more noncontiguous lobes were classified as diffuse. A miliary pattern was denoted separately when present. The location of the infiltrate was specified as upper, middle (lingular), or lower lobe. Cavities were differentiated from pneumatoceles by wall thickness, the cutoff being 3 mm. When interpreting the radiology report, the above features were recorded as absent when not specifically remarked on.

RESULTS

Demographics

We identified 164 patients with pulmonary TB, 20 (12%) of whom were also infected with HIV. The average age of the HIV-infected patients was 40 years (range, 27 to 56 years), compared with 51 years (range, 12 to 91 years) in the non-HIV-infected patients. All HIV-infected patients and the substantial majority (82%) of non-HIV-infected patients were male. Review of public health records demonstrate that these findings are consistent with those generated throughout the state of Washington before, during, and after the study period.

Chest radiographs were available for 17 of the 20 HIV-infected patients with TB; radiographs for two patients had been destroyed, and those for another were not available for review. During the study period, there were 880 HIV-infected patients admitted to the hospital with pneumonia of other causes. We randomly selected 40 age- and sex-matched patients from this group for comparison. Records

were available for 37 of these patients who had an average age of 39 years (range, 26 to 60 years).

Mycobacteriology

Four hundred ninety-five sputum specimens from 164 patients were submitted to the laboratory for evaluation. Cultures were performed on 493 of these specimens with an overall sensitivity of 93.5% (461/493). Patients were presumed to be receiving antituberculous chemotherapy if a positive culture had been reported at least 1 week prior to submission of subsequent specimens. Excluding these specimens, the overall sensitivity of the culture increased to 99% (461/467). Culture of the initial sputum specimen was positive in all 20 HIV-infected patients and in 99% of non-HIV-infected patients (142/144).

Seventy percent of all HIV-infected patients (14/20) and 71% of all non-HIV-infected patients (103/144) had at least one positive smear (Fig 1). The concordance of serial smears was high as demonstrated by the fact that a similar proportion of all specimens were positive: 68% (41/60) in HIV-infected patients and 70% (304/435) in the non-HIV-infected patients. The discordance that did occur involved the initial smear. Three of the 14 HIV-infected and 11 of the 103 non-HIV-infected patients who were ultimately smear positive were negative on the initial smear. The first smear identified the majority of HIV-infected patients (79%) and non-HIV-infected patients (90%) who were ultimately smear positive. However, the sensitivity for the diagnosis of TB dropped to 55% and 64%, respectively, when only the first smear was considered.

Only 44 of the 61 patients (72%) with negative initial smears had subsequent sputum smears. Three of five second smears obtained in HIV-infected patients were positive while 10 of 39 were positive in the non-HIV-infected patients. One of eight third smears was positive in a non-HIV-infected patient while a single negative smear was obtained in an HIV-infected patient. HIV-infected patients who were initially smear negative were less likely to have a second sputum smear obtained than their non-HIV-infected counterparts (5/9 [56%] and 39/52 non-HIV-patients [75%], respectively). However, they were more likely to have undergone a BAL following a negative sputum smear (2/9 [22%] and 3/52 [6%], respectively). Most HIV-infected and non-HIV-infected patients (8/9 [89%] and 42/52 [81%], respectively) who had a negative initial smear but no subsequent sputum smears did have another study (BAL, pleural fluid analysis, pleural biopsy, or lung biopsy) looking for *M tuberculosis*.

Mycobacterium avium Complex

MAC was recovered from the respiratory tract of 106 HIV-infected patients during the study period. Although the pathologic significance of the respiratory isolates was not addressed, 55 (52%) of these patients had a diagnosis of disseminated MAC (defined by positive blood cultures) at the time of, or before, sputum isolation. There were 147 smears generated in these 106 patients, 19 of which were positive for AFB. These 19 smears were generated in 13 patients, representing an overall smear sensitivity of 12% for MAC in the lung. Twenty-seven HIV-infected patients had a positive AFB smear during

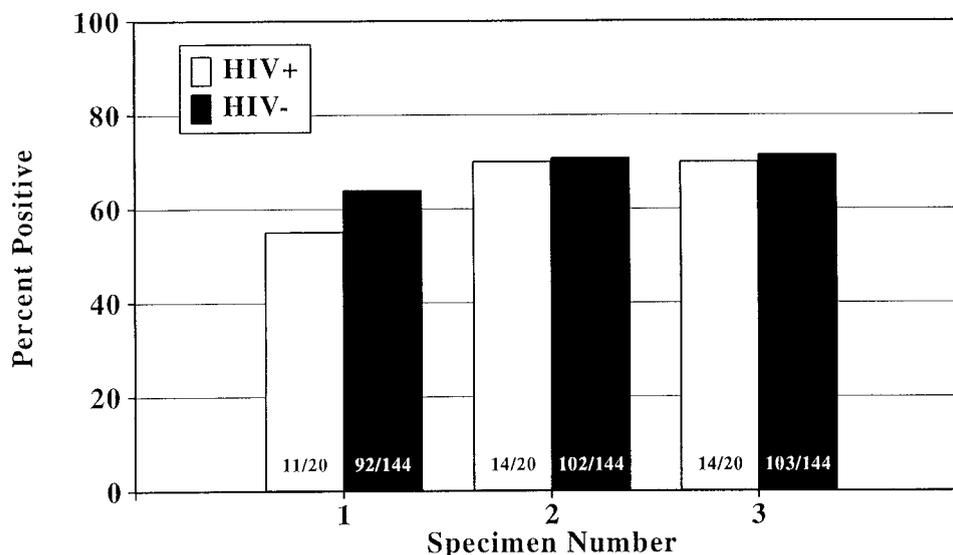


FIGURE 1. Diagnostic yield of serial smears. The cumulative yield in HIV-positive and HIV-negative patients is shown.

the study period, 14 *M tuberculosis* and 13 MAC, for an overall specificity of 52%. *M tuberculosis* was not identified in any of the 106 patients who were infected with MAC.

Smear Quantitation

The quantitative analysis of the sputum smear for all TB patients in this study is presented in Table 1. When considering all positive smears, HIV-infected and non-HIV-infected patients demonstrated the same number of organisms by American Lung Association scoring of the smear (2.36 ± 0.63 and 2.31 ± 0.71 , respectively). Patients who tested positive on the initial smear had significantly more organisms than those who were positive only on subsequent smears (2.37 ± 0.69 vs 1.73 ± 0.64 [$p=0.005$]). This trend was observed in both groups, although the difference achieved statistical significance only in the non-HIV-infected patients (2.36 ± 0.70 vs 1.60 ± 0.52 [$p<0.005$]). HIV-infected patients who ultimately grew MAC had fewer organisms on smear than those who grew *M tuberculosis* (1.95 ± 0.52 vs 2.45 ± 0.52 [$p<0.05$]). Eighty-nine percent (17/19) of the smears that identified MAC were positive at either the 1+ or 2+ level. In contrast, only 41% of positive smears related to *M tuberculosis* infection were positive at the 1+ or 2+ level.

Radiography

The comparison between chest radiographic findings in HIV-infected patients with TB and those with pneumonia of other causes is presented in Table 2. Diffuse infiltrates were the most common radiographic finding, being present in 76% of patients with TB and 65% of those with other forms of pneumonia. Focal upper lobe disease was not observed in the TB group and was present in only 5%

Table 2—Radiographic Findings in HIV-Infected Patients With TB and Pneumonia of Other Causes

	HIV+ Patients With TB, No. (%) (n=17)	HIV+ Patients With Non-TB Pneumonia, No. (%) (n=37)
No infiltrate	2 (12)	5 (14)
Focal infiltrate	2 (12)	8 (22)
Upper lobe	0	2 (5)
Middle or lower lobe	2 (12)	6 (16)
Diffuse infiltrate	13 (76)	24 (65)
Upper lobe	2 (12)	0
Middle or lower lobe	6 (35)	5 (14)
Interstitial pattern	5 (29)	19 (51)
Miliary pattern	1 (6)	0
Cavitation	3 (18)	0
Miliary pattern or cavitation	4 (24)	0
Adenopathy	4 (24)	4 (11)
Effusion	3 (18)	6 (16)

of the comparison group. Two TB patients had upper lobe involvement as part of a more diffuse abnormality. Eighteen percent of patients with TB had pleural effusions compared to 16% in patients with other forms of pneumonia. There was significant interobserver variability in the interpretation of adenopathy such that a tiebreaking interpretation was required in 35% of TB patients and in 41% of the comparison group. Evaluation for adenopathy was not possible because of adjacent radiographic densities in 35% of patients with TB and 16% of patients with pneumonia of other cause. Despite these difficulties, adenopathy was slightly more common in patients with TB [4/17 [24%]] than in patients with pneumonia of other causes (4/37 [11%]), although this difference did not achieve statistical significance ($p=0.29$). The presence of cavitation or a miliary pattern were the only variables that discriminated between the two groups. All four of the patients who demonstrated either of these findings had TB ($p=0.014$).

Table 1—Quantitative Analysis of Positive Sputum Smears*

Positive Smears	HIV+ Patients			HIV- Patients		
	n	Mean	SD	n	Mean	SD
<i>M tuberculosis</i>						
All smears	14	2.36	0.63	113	2.31	0.72
First smears	11	2.45	0.52 } [†]	102	2.36	0.70 } [†]
Second smears	3	2.0	0.82 } [†]	10	1.60	0.52 } [†]
MAC						
All smears	13	1.95	0.52 [§]			

*A quantitative analysis in HIV-positive and HIV-negative TB patients is shown. The numbers shown (mean and SD) were generated using the American Lung Association scoring system.

[†]Not significant.

[‡] $p<0.005$.

[§] $p<0.05$ (when comparing all positive smears for TB with those for MAC).

DISCUSSION

The microscopic examination of expectorated sputum provides a rapid and inexpensive means by which the diagnosis of TB can be established. This study adds to the list of those demonstrating that the sputum smear is as sensitive for the diagnosis of TB in HIV-infected patients as it is in non-HIV-infected patients.¹³⁻¹⁷ The sensitivity (70%) that we report is consistent with, if not superior to, that reported in other studies^{5,6,17,18} despite the fact that three smears were generated in the minority of our patients. Had we only considered the first sputum specimen, the smear would have established an early diagnosis in 55% of the HIV-infected patients and 65% of the non-HIV-infected TB patients. We would have failed to isolate three HIV-infected and 13 non-HIV-infected patients during the study period. However, the overall diagnostic yield would not have dropped appreciably since culture of the initial specimen demonstrated the *M tuberculosis* in all (20/20) of the HIV-infected patients and 99% (142/144) of the non-HIV-infected patients.

The sputum smear is the only means available to assess the public health risks posed by patients suspected of having TB. The utility of a single sputum smear in making this determination hinges on the risk of disease transmission in patients who would have been identified on subsequent smears. Unfortunately, to our knowledge, no studies have been performed to specifically address this issue. However, historical data provide some insights. Studies conducted on the household contacts of TB patients demonstrate that the incidence of skin test conversion varies directly with the sputum smear status of the index case; those exposed to smear-negative patients do not appear to be at increased risk for the development of latent infection with *M tuberculosis*.^{19,20} These studies were conducted using the Ziehl-Neelsen stain. The auramine stain that is in common use today increases the sensitivity of the sputum smear by 18%.⁶ Had this stain been used in these early studies, 18% of the patients identified as smear negative would have been positive. These studies would then support the concept that patients with *few* (detectable only by auramine) or no organisms in their sputum do not pose an important public health threat. Since fewer organisms were observed when patients were identified on smears other than the first, these patients may be less infectious. This interpretation would shed a more favorable light on the use of a single sputum smear.

We acknowledge that, while a single negative sputum smear makes the diagnosis of TB significantly less likely, these data do not support a single specimen as the sole means of assessing the public

health risks posed by patients suspected of having TB. However, the addition of a second sputum smear identified all HIV-infected patients and all but one non-HIV-infected patient who were ultimately identified to be smear positive. In our patient population, it would appear that two smears, rather than the traditional three, are sufficient to make this determination.

Despite the fact that MAC is an important pathogen in AIDS patients, it is a relatively uncommon respiratory pathogen in this patient population.²¹ It is, however, commonly isolated from the respiratory tract of AIDS patients and when seen on smear, can lead clinicians to falsely conclude that their patients are infected with *M tuberculosis*. We identified 13 patients with positive sputum smears that were subsequently attributed to MAC. Two clinical variables distinguished these patients from those with TB. Half of them had disseminated MAC at the time of sputum collection and there were significantly fewer organisms present on the sputum smear in those who ultimately grew MAC. When considering positive smears attributable to MAC, the overall specificity of the sputum smear for *M tuberculosis* in HIV-infected patients dropped to only 52%.

Since it was clear that the sputum smear would be an imperfect diagnostic test for the presence of TB in HIV-infected patients, we also addressed what help the radiographic findings at the time of presentation might be in identifying these patients. We found that there was no distribution of infiltrate that was specific for the diagnosis of TB in this patient population. Hilar adenopathy has been proposed as an important radiographic variable in the evaluation of TB in this patient population.^{13-15,22} We were unable to confirm this finding. In fact, our observations suggest that this adenopathy is too subjective a finding to be relied on in clinical decision making. In this small study, the findings of cavitation or a miliary pattern were highly specific for the diagnosis of TB. However, in the absence of these findings, the chest radiograph was not helpful in differentiating between TB and pneumonia of other causes.

The limitations of currently available technology mean that there will always be some uncertainty and, therefore, risk associated with the evaluation and care of patients suspected of having TB. The question for clinicians, infection control practitioners, and hospital administrators is what level of risk is acceptable. The answer to this question is multifactorial and is likely to be different for every institution that considers the problem. The prevalence of TB, the prevalence of drug resistance, and the availability of reliable mycobacterial data are but a few of the local variables that should impact this decision. Accordingly, large prospective trials should be performed

before fewer than three smears are routinely employed in the evaluation of patients suspected of having TB.

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